REMARKS

Applicants have presented evidence as seen in Exhibits A-K filed with the Response of October 17, 2002 and in the ClustalW alignment faxed to Examiner Davis on January 28, 2003, that HPAK shares homology with the kallikrein polypeptide family, a family consisting of members known to have undisputed utility, and therefore, homology can and is being used to show a substantial likelihood that the claimed polypeptide is similarly useful. Applicants need not show any more to demonstrate utility. Applicants provide herewith explanations of selected Exhibits, their findings and interpretation of evidence disclosed, in support of the utility of HPAK.

A. Exhibit A

Exhibit A is a BlastP comparison of SEQ ID NO:1 (the "Query" sequence) against the Genpept version 131 protein sequence database from the National Center for Biotechnology Information, division of the National Library of Medicine at the National Institutes of Health, Bethesda, MD.

The top 20 polypeptide sequences having sequence homology to SEQ ID NO:1 are listed along with their individual "Score" and "E-value." The E-value indicates the probability of obtaining the observed polypeptide sequence alignment by chance. The first seven sequences producing significant alignments have an E-value of 1.0 e-134. Of the top 20 sequences, all are proteases, belonging to the kallikrein family, specifically the kallikrein identified as kallikrein 11, also known as hippostasin, and also known as trypsin-like serine protease (TLSP, PRSS20) (*infra*).

Each alignment represents the amino acid residues found in both SEQ ID NO:1 ("Query") and also present (identical residues presented in the middle line of each 3-line alignment, read vertically) or representative of a conservation substitution ("+" present in the middle line of each 3-line alignment, read vertically) in each of the 20 sequences producing significant alignments. For example, examination of the first alignment shows a comparison of SEQ ID NO:1 (Query) to g8574439 (Sbjct = subject). The amino acid residues of comparable identity begin with residue V5 of SEQ ID NO:1 and L38 of g8574439, identified as prostate-type hippostasin [Homo sapiens] (numbering to the left of the alignment). Beginning at residue L61 of SEQ ID NO:1 and L90 of g8574439 (numbering to the right

of the alignments), one of ordinary skill in the art understands that the two sequences are 100% identical to the end of each sequence, i.e., N253 of SEQ ID NO:1 and N282 of g8574439.

Applicants also bring to the Examiner's attention that relationships can also be made among the 20 individual sequences producing significant alignments. First, there appears to be two known isoforms of kallikrein 11, one consisting of 250 residues as seen in GI 6681454, GI 5713132, GI 18314498, GI 11244769 and GI 10799396, and one consisting of 282 residues, GI 8574439 and GI 3649791. In the papers by Mitsui, S. et al., Exhibit G (page 207), and Nakamura, T. et al., Exhibit H (pages 72-74), the two isoforms of kallikrein 11 are identified. These two isoforms are a result of alternative splicing, which is regulated in an organ-specific manner. The 250 residue isoform is found in brain tissue, with the prostate-type having an additional 32 residues at the N-terminus (Nakamura et al. page 73).

Secondly, each of the sequences representing isoform 1 having 250 residues in length, and the two sequences of isoform 2 having 282 residues in length, are 100% identical to each other within the mature peptide region, G25 to N250, numbering on the 250 residue isoform, which corresponds to residues G28 to N253 on SEQ ID NO:1. This 100% sequence identity was illustrated in the alignment faxed to Examiner Davis on January 28, 2003. Thus, the discoveries of individual investigators about one sequence isoform are applicable to the other isoform.

B. Exhibit B

Exhibit B shows the GenBank file of an illustrative example of one homolog of SEQ ID NO:1, kallikrein 11 (GI 18314498).

C. Exhibit C

Serine protease enzymes, including the instant invention of SEQ ID NO:1, are further identified by functional motifs, domains and sites indicative of biological activity. Exhibit C represents the likely trypsin-like serine protease (Tryp_SPc) and trypsin (trypsin) domains within SEQ ID NO:1.

The first alignment is SEQ ID NO:1 (Query; residues R21 to I243) aligned with a representative trypsin-like serine protease domain comprising 230 amino acid residues (Sbjct, gnl|CDD|7285). The second alignment is SEQ ID NO:1 (Query; residues R22 to I243) aligned with a

representative trypsin domain comprising 217 amino acid residues (Sbjct, gnl|CDD|7446). One of ordinary skill in the art would interpret the identification of both trypsin-like serine protease and trypsin domains within SEQ ID NO:1 as evidence that more likely than not, SEQ ID NO:1 would also have protease activity because SEQ ID NO:1 has the functional domains necessary for protease activity.

D. Exhibits F, G and H

Exhibits F-H provides evidence of SEQ ID NO:1 functioning as a serine protease as well as demonstrating differential protein expression patterns in prostate cancer verses noncancerous prostate tissue.

Exhibit F contains the GenBank file for human trypsin-like serine protease (GI 5713131, 250 residues in length, the 3rd sequence producing a significant alignment with SEQ ID NO:1 in Exhibit A). Additionally, the manuscript publishing the genomic organization, chromosomal mapping, tissue expression and hormonal regulation of trypsin-like serine protease (TLSP PRSS20) (GI 5713131), also known in the art as kallikrein 11, by Yousef, G.M. et al., (2000) Genomics 63: 88-96, is included.

Turning to the Yousef et al. paper, one sees that on page 91, 2nd column, first full paragraph, TLSP is characterized as a kallikrein which is synthesized as a pre-proenzyeme with a N-terminal signal peptide, the prezymogen, followed by an activation peptide and the enzymatic domain located at the C-terminus. TLSP is found, when aligned to other kallikreins, to contain the catalytic triad of serine proteases, His62, Asp110, and Ser203 (see Figure 5, page 93). The same catalytic triad is also found in SEQ ID NO:1 at His65, Asp112 and Ser206. One of skill in the art would find the presence of the catalytic triad evidence that more likely than not that SEQ ID NO:1 would also have serine protease activity.

Exhibit G contains the GenBank file for prostate-type hippostasin (GI 8574439, 282 residues in length, the 1st sequence producing a significant alignment with SEQ ID NO:1 in Exhibit A). Additionally, the manuscript publishing the identification of GI 8574439, termed TLSP/hippostasin (PRSS20) by Mitsui, S. et al. (2000) Biochem. Biophys. Res. Comm. 272:205-211 is provided.

Mitsui et al. teach the construction of a recombinant TLSP protein in a baculovirus expression system. Using residues I54-N282 (corresponding to I25-N253 in SEQ ID NO:1) in the construct, enzymatic activity was observed as seen by hydrolysis of synthetic peptides known to be kallikrein

substrates (Mitsui et al. p. 208-209). Mitsui et al. provide scientific evidence of the proteolytic activity of kallikrein 11 (TLSP). Thus, one of skill in the art would also conclude more likely than not that such activity could also be asserted for SEQ ID NO:1 in view of the high level of sequence homology between SEQ ID NO:1 and kallikrein 11 (KLK11).

Exhibit H contains the manuscript of Nakamura et al. (Nakamura, T. et al. (2001) Prostate 49:72-78) which describes the tissue expression pattern of the two isoforms of hippostasin (PRSS20/KLK11) in prostate cancer cell lines. Additionally, Nakamura, T. et al. have found that all prostate cancer cell lines tested expressed only isoform 1 (brain-type, 250 residues in length) and not isoform 2 (prostate-type, 282 residues in length), while both normal prostate and benign prostatic hypertrophy (BPH) tissues expressed both isoforms (Nakamura, pages 75-76). Nakamura, T. et al. conclude that hippostasin, the brain-type isoform-1 may be used as a marker to distinguish prostate cancer and BPH Therefore, one of skill in the art would find SEQ ID NO:1 similarly useful as a diagnostic marker for prostate cancer.

E. Exhibit J

Exhibit J contains the GenBank file for human keratinocyte trypsin-like serine protease (GI 11244769), 250 residues in length, the 6th sequence producing a significant alignment with SEQ ID NO:1 in Exhibit A). Additionally, the manuscript publishing the sequencing and expression analysis of the serine protease gene cluster located on chromosome 19q13 by Gan, L. et al., (2000) Gene 257:119-130, is included, this paper was faxed to Examiner Davis on January 28, 2002.

The analysis of the serine protease genes as presented in Figure 3 (SEQ ID NO:1 is represented as TLSP, Gan, page 126) indicate that the serine proteases have hydrophobic signal peptides of 16 to 33 residues, suggesting they are secreted serine proteases. Following the signal peptide is a short activating peptide and then a protease domain of varying length. There is 30-40% sequence conservation among these proteases. However, these serine proteases share 41 amino acids in their protease domains (black = conserved residues, gray = similar residues, Figure 3) including the catalytic triad *supra*, many of the disulfide bridges and residues lining the "enzymatic active pocket. Gan et al. further note that TLSP has an insertion of residues prior to the catalytic Asp (D) residue

which forms the "kallikrein loop" which is believed to interact with the substrate and thus determine kallikrein enzymatic specificity (Gan, page 125, 2nd column, 2nd paragraph).

The kallikrein loop of SEQ ID NO:1 based on the alignment of TLSP in Figure 3 of Gan et al. appears to be residues P106-D109, PNKD. Based on the high level of conservation between SEQ ID NO:1 and TLSP (kallikren 11) and the presence within SEQ ID NO:1 of the identical kallikrein loop as that of TLSP, one of ordinary skill in the art would conclude that more likely than not, SEQ ID NO:1 would have the same substrate specificity as TLSP.

F. Exhibit K

Exhibit K contains a review paper of the human tissue kallikrein gene family, examining the structure, function and disease association of kallikrein genes (G.M. Yousef and E.P. Diamandis, (2001) Endocrine Rev. 22:184-204). The catalytic triad has maximum sequence homology between all 15 members of the kallikrein gene family. The highly conserved regions around the catalytic triad are: WVLTAAHC, DLMLL, and GDSGGPL (Yousef and Diamandis, pages 187-188). Examination of TLSP's and SEQ ID NO:1's residues surrounding the catalytic triad reveal comparable conservation as well. For TLSP; WLLTAAHC, DIMLV, GDSGGPL and for SEQ ID NO:1; WFLTAAHC, DIMLV, GDSGGPL.

is L or F conservative substit. of V ?

Catalytic Triad: WVLTAAHC, DLMLL, GDSGGPL

Examination of the second position of the H65 region suggests variability between the otherwise "highly conserved" regions, as neither SEQ ID NO:1 nor TLSP has the "V" (valine) residue. However, the "F" (phenylalanine) residue present in SEQ ID NO:1 is a recognized conservative amino acid substitution for "L" (leucine) present in TLSP. In all other instances, SEQ ID NO:1 and TLSP follow the conserved sequence pattern for the kallikrein gene family. Thus, one of skill in the art would conclude that more likely than not that SEQ ID NO:1 is yet another member of the kallikrein gene family, and more likely than not yet another splice variant of kallikrein 11.

Kallikrein 11 has been established by several investigators to be a functioning serine protease enzyme with differential protein expression in cancerous prostate tissue versus normal and BPH prostate tissues. Consequently, SEQ ID NO:1 would be understood by one of ordinary skill in the art to also be a serine protease, associated with cancerous prostate tissue and useful in the diagnosis of

prostate cancer as discussed *supra*. Therefore, SEQ ID NO:1 more than fulfills the utility requirements of the Patent Office and withdrawal of the rejections under 35 U.S.C. §§ 101 and 112, first paragraph is hereby requested.

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CONCLUSION

In light of the above amendments and remarks, Applicants submit that the present application is fully in condition for allowance. Early notice to that effect is earnestly solicited.

If the Examiner contemplates other action, or if a telephone conference would expedite allowance of the claims, Applicants invite the Examiner to contact the undersigned.

Applicants believe that no fee is due with this communication. However, if the USPTO determines that a fee is due or that an excess fee has been paid, the Patent Office is authorized to debit or credit (respectively) Deposit Account No. **09-0108**.

Respectfully submitted,

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